<Division of Biochemistry> Biofunctional Design-Chemistry

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- Biofunctional Design-Chemistry -

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Visitors

Prof VIVES, Eric	University of Montpellier II, France, 16 February 2004
Dr MIHALA, Nikolett	Research Group for Peptide Chemistry, Hungarian Academy of Sciences, Hungary, 27 July - 31 August 2004
Prof DIBO, Gabor	Institute of Chemistry, Eotvos L. University, Hungary, 22 October 2004
Prof HUDECZ, Ferenc	Research Group for Peptide Chemistry, Hungarian Academy of Sciences, Hungary, 23 October - 4 November 2004
Prof MICHEL, L. J. Sarah	Maryland University, USA, 16 November 2004

Scope of Research

As an interface of chemistry and biology, this division investigates the molecular mechanism of specific interaction between biologically active molecules and macromolecular receptors. We also aim at the design of novel functional molecules. Current research subjects are as follows: (1) Elucidation of the DNA recognition mode of C_2H_2-type zinc finger proteins and design of artificial DNA binding peptides. Studies on the structure and function of a zinc finger motif by coordination of a metal. (2) Design and synthesis of artificial functional peptides and development of novel intracellular delivery systems aiming at elucidation and control of cellular functions.

Research Activities (Year 2004)

Presentations

Artificial Zinc Finger Proteins: Designs and Functions, Sugiura Y, Special Seminor of Risbon University, Risbon, Prortogal, 15 March.

Structure and Function of Zinc Finger Proteins, Sugiura Y, 124th Annual Meeting of Pharmaceutical Sciences of Japan, Osaka, 29 March.

Delivery of macromolecules into cells using non-viral vectors, Arginine-rich peptides: aspects of membrane translocation, Futaki S, Molecular design in drug delivery and development symposium series, Toronto, Canada, 9 - 10 July.

Creation and Function of Zinc Finger Proteins, Sugiura Y, 31st Organi Reaction Meeting, Kyoto, 31 July.

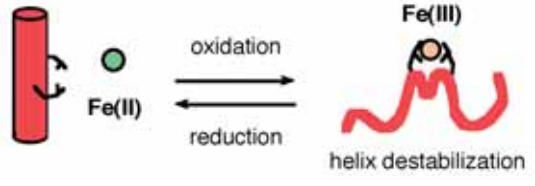
Grants

Sugiura Y, Role of multi-zinc fingers in gene expression and creation of their architectures, Grant-in-Aid for Scientific Research (B) (2), 1 April 2002 - 31 March 2005.

Sugiura Y, Creation and DNA binding of the longest

Metal-mediated Modulation of Peptide Structure and Recognition

Helical peptide segments that alternate their conformation in accordance with external stimuli are attractive building blocks for the development of novel peptide devices and materials. Substantial efforts have been focused on stabilizing the helical structure by metal chelation and ion pair formation. However, approaches to destabilize peptide structures may be promising for the helices having a strong tendency of helix formation. We have prepared helical peptides equipping a pair of the iminodiacetic acid derivatives of lysine (Ida), and showed the importance of the topologies of the Ida residues for helix stabilization and destabilization. For the preparation of Ida-containing peptides, we developed a novel approach using the direct conversion of Lys to Ida on the Fmoc-solid phase resin. When the Ida residues were placed at i and i+2 positions in a 17-redsidue helical peptide, the addition of Fe(III) resulted in a significant decrease in the helical content whereas Fe(II) had no influence on the helix stability. The possibility of redox control of the helical structure was then exemplified by the reduction of Fe(III) to Fe(II) using $Na_2S_2O_4$ followed by the subsequent reoxidation. Mutual recognition between the transcription factor Jun-derived leucine-zipper peptide segment and the Fos-derived one containing Ida residues was also modulated in the presence of Fe(III). The concept of structural switching by helix destabilization would open new avenues for the design of novel peptide-based functional molecules and devices.



Exchange of Histidine Spacing between Sp1 and GLI Zinc Fingers: Distinct Effect of Histidine Spacing-Linker Region on DNA Binding

A C_2H_2-type zinc finger has a tandemly-repeated structure, which consists of independent modules with the consensus sequence, ((Tyr, Phe)-X-Cys-$X_{2,4}$-Cys-X_3-Phe-X_5-Leu-X_2-His-$X_{3\text{-}5}$-His-$X_{2\text{-}6}$). Each domain forms a compact bba structure held together tetrahedrally by coordination of a zinc ion with two invariant cysteines and histidines. Typical C_2H_2-type zinc fingers recognize the three-base-pair subsite mainly on one strand using key amino acid residues of the a-helix. Based on these features, new zinc fingers with various sequence specificities have been designed by mutating amino acid residues in the a-helix by rational structural design and by a phage-display-based method. In the DNA recognition mode of C_2H_2-type zinc fingers, the finger-finger connection region, consisting of the histidine spacing and linker, would be important for determining the orientation of the zinc finger domains. The histidine spacing is conserved from HX_3H to HX_5H and has various conformations in accordance with the number of amino acid residues. On the basis of the previous structural analyses, an HX_3H-type spacing forms a 3_{10}-helix, whereas HX_4H-type and HX_5H-type spacings form helical structures. The local conformational alteration of the histidine spacing might result in changing the DNA binding of zinc finger proteins. In order to clarify the influence of spacing between two ligand histidines in the DNA binding, we exchanged the histidine spacing between Sp1 and GLI zinc fingers, which have an HX_3H-TGEKK linker (typical) and an HX_4H-SNEKP linker (atypical), respectively (Figure 1). A significant decrease in the DNA binding affinity and specificity is found in Sp1-type peptides, whereas GLI-type peptides show a mild reduction. To evaluate the effect of the linker characteristics, we further designed Sp1-type mutants with an SNEKP linker. As a result, the significant effect of the histidine spacing in Sp1-type peptides was reduced. These results demonstrate that (1) the histidine spacing significantly affects the DNA binding of zinc finger proteins and (2) the histidine spacing and the following linker regions are one effective target for regulating the DNA recognition mode of zinc finger proteins.

Figure 1: Swapping of histidine spacings between Sp1 and GLI zinc fingers.

multi-zinc finger protein, Sp1ZF15, Grant-in-Aid for Exploratory Research, 1 April 2004 - 31 march 2006.

Futaki S, Functional design of cell-targeting peptides, PRESTO program, Japan Science and Technology Agency,1 November 2002 - 31 October 2005.